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# Study on Solvent Sublation for Separation and Enrichment of Total flavonoids in *S. m edusa*. Maxim

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Abstract：A novel method for separating and enriching total flavonoids in *S. m edusa*. Maxim by solvent sublation and determining the content by UV spectrophotometry has been developed. The influences of sublation solvent，nitrogen flow rate，pH of solution，sublation time and electrolyte etc. on the sublation efficiency of total flavonoids were investigated in detail, and the optimal conditions of the solvent sublation were obtained. The proposed method was used to determine the contents of total flavonoids in *S. m edusa*.. Sample content was 17.276mg/g under the optimal condition and RSD was 1.95%.The results show that the method is satisfactory.



Key Words:Solvent sublation；spectrophotometry；total flavonoids；*S. m edusa*. Maxim

*S.m edusa*. Maxim is an Asteraceae Saussurea DC plants, which is common herbs in China. It possesses efficacies of invigorating circulation of blood, warm uterus, eliminate blood stasis, get rid of cold and moisture in the body, and **s**trengthen body, etc..*S.m edusa*. Maxim contains flavonoids, alkaloids, lactones sterols, polysaccharides and a variety of volatile oil chemical constituents, including one of the major secondary metabolites of flavonoids and flavonoid glycosides. Modern pharmacological studies have shown that the *S.m edusa*. Maxim has anti-inflammation, anti-tumor, anti-early pregnancy, anti-aging, anti-fatigue and the role of scavenging free radicals, etc.[1] .

Currently, the methods for determining total flavonoids in *S. m edusa*. Maxim are colorimetric methods [2-4], HPLC [5], etc. and the extraction methods of total flavonoids from natural *S. m edusa*. Maxim are water extraction, a solvent extraction and microwave extraction [3]. The solvent sublation which is a novel technology of separation and enrichment has been developed in the last 20 years, which have the characteristics of completion the separation and enrichment both, so trace amounts of metal ions and organic pollutants in water have been measured by solvent sublation in many literatures published [6].At present, there are some active ingredients of traditional Chinese medicine has been effectively separated using the method [7-9], but the method used for separation and determination of total flavonoids of *S. m*

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*edusa*. Maxim has not been reported. In this paper,a novel method for separating and enriching total flavonoids in *S. m edusa*. Maxim has been conducted due to hydrophobic and nitrogenphilic of the total flavonoids in *S. m edusa*. Maxim. In the sublation process, the flavonoids in the aqueous phase can be adsorbed by the "gas - liquid" interface produced through a lot of bubbles and raised to the sublation columns top with air bubbles. The flavonoids dissolved in the upper organic phase after the bubble burst, and then measure the absorbance of organic phase, which can obtained the total flavonoid content. For the rational development and make full use the resources of the *S. m edusa.* Maxim, the optimum enrichment conditions of the total flavonoids in the *S. m edusa*. Maxim by the solvent sublation were obtained according to the total flavonoid content as the indexes.The results show that this method is satisfactory.

## 1 Experimental

### *1.1 Instruments and reagents*

TU1901 double-beam UV-Vis spectrophotometer (Beijing Puxi General Instrument Co., Ltd.), MC756 UV-Vis spectrophotometer (Shanghai), Sartorius (PB-10) pH meter, Mettler Toledo AL204 electronic balance, solvent sublation column (G4) (self-made), see reference [10]. Nitrogen gas, NaOH, NaC1, HC1, n-butanol, ethyl acetate, petroleum ether, toluene were analytical grade, standard sample rutin was purchased from Chengdu Manste, *S. m edusa*. Maxim samples were purchased from Xining pharmacies, water was distilled water.

### *1.2 Experimental Methods*

The air-dried coarse powder of the plant (6.3686g) were heated reflux and extracted with 70% EtOH (100mL × 2, each extraction lasted 50min ).The sample stock solution was prepared by filtrating and combining filtrate, then diluting to the mark in a 250mL volumetric flask with distilled water. A 10mL stock solution was transferred to a 500mL beaker and diluting to 300mLwith distilled water. Then the pH of the solution was adjusted to roughly 3 with 0.1mol $L^{-1}$ hydrochloric acid solution and 0.1mol $L^{-1}$ sodium hydroxide solution. The solution was mixed well and transferred to a flotation column, and then 10.00mL n-butanol was added. The working solution were floated by bubbling nitrogen at a flow rate of 60 ~ 70mL$min^{-1}$ from the bottom of the column for 40min, then the working solution were deposited for a short while, and sucked out the upper organic phase 10mL of on the surface of the aqueous solution, then diluting to the mark in a 10mL volumetric flask with n-butanol.The analytes in the organic phase were determined by visible spectrometry. The wavelength measured was 360nm for analytes.

## 2 Results and discussion

### *2.1 Absorption curve*

The experiment was performed according to the procedure given in the experimental section 1.2, and the sample solution was extracted by solvent sublation and the absorbances of the analytes concentrated in *S. m edusa*. Maxim are measured by the UV absorption spectra in the range 200 ~ 400nm. The absorption peaks located at 220 and 360nm and the 360nm wavelength was choiced for the quantitative analysis to determine the absorbance for evaluating the efficiency of sublation.

### *2.2 The choice of sublation solven***t**

Butanol, ethyl acetate, toluene and petroleum ether were selected to as sublation solvent according to experimental methods 1.2. The results showed that n-butanol was the best sublation solvent.

### *2.3 The best choice of pH*

The pH of the sample solution was adjusted to in the range of 1-8 with 0.1mol $L^{-1}$ hydrochloric acid solution and 0.1mol $L^{-1}$

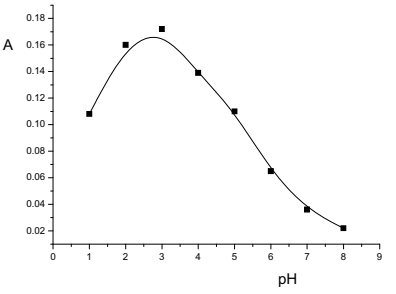


Fig.1 Effect of pH

sodium hydroxide solution, according to experimental methods 1.2. The results (Fig.1) shows that the solution pH=3 was the best condition.In this pH, the flavonoids in the solution is the molecular state with good hydrophobic, so especially suitable for solvent sublation.

*2.4 The influence of nitrogen flow rate*

The bubbling of gas is very important in every flotation method because the bubbles float the analyte to the surface of the solution. As the bubbles rise through the gas diffuser, a surfactant bound with the flavonoids is adsorbed at the gas–liquid interface. When the bubbles reach the liquid–liquid interface, they are unable to overcome the interfacial tension immediately. Generally, the rate of gas–liquid interfacial area generation can be increased by increasing the gas flow rate. However, it is recommended to avoid gas flow rates that are too high because of turbulent mixing at the solvent-aqueous solution interface. When the nitrogen flow rate exceeded a certain critical value, the bubble will rise along the wall of the column and burst in the gas–liquid interface, and the bubble carried the flavonoids will return directly into the water phase, but not into the organic phase, which is not benefit to sublation. After various experimental results (Fig.2), the flow rate of nitrogen was fixed at 60~70mL$min^{-1}$ for this experiment.

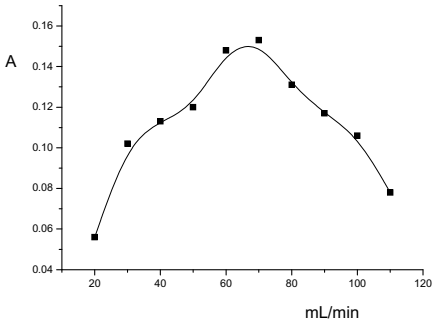


Fig.2 Effect of nitrogen flow rate

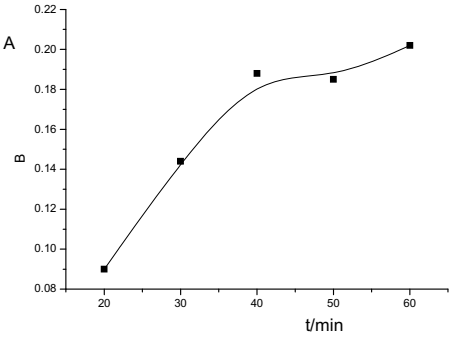


Fig.3 Effect of sublation time

*2.5 Flotation time*

Flotation time is an important parameter. As is shown in Fig.3, the absorbance gradually increased with increasing flotation time up to 40 min. After 40min the absorbances were almost constant. The flotation time was fixed at 40 min in this experiment.

*2.6 The influence of the electrolyte*

Different amount electrolyte NaCl was added in the sublation solution. The results show that efficiency has little effect, so electrolyte was not necessary.

*2.7 Calibration Curves*

The calibration curves were prepared with the standard rutin solutions which were treated under the experimental conditions stated above 1.2, a certain concentration of standard solution 0, 5.0, 10.0, 15.0 and 20.0mL were pipetted separately. After linear regression, the regression equation obtained was A = 3.0464 C-0.0046, R = 0.9992 (n = 5), where Y is the absorbance and X is the rutin mass concentration.

*2.8 Sample Analysis*

According to experimental method 1.2, the content in the samples were determined under the optimized experimental conditions stated above. The results are 17.276mg / g (n = 5) and RSD is 1.95%. It can be seen that the proposed method is more simple and quick, and thus very suitable for the extraction and determination of total flavonoids in *S. m edusa*. Maxim.

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